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Procedia Environmental Sciences 33 (2016) 70 – 77

Procedia
Environmental Sciences

The 2nd International Symposium on LAPAN-IPB Satellite for Food Security and Environmental Monitoring 2015, LISAT-FSEM 2015

Influence of agro-ecology on growth and performance of several potential mutants of cassava

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Abstract

Cassava (*Manihot esculenta*) is an important source for food, feed, and raw material for industries in Indonesia. In order to develop new high yielding and high starch content varieties, two national varieties (i.e. Adira-4 and Malang-4) and introduced variety (UJ-5) were irradiated with gamma ray to induce genetic variety. Evaluation of potential cassava mutants was needed to learn their growth and performance in two different agro ecologies, which were acid upland and optimum upland, in order to select adaptive mutants. Cuttings from sixteen mutants and three parent lines (UJ-5 (V3), Adira-4 (V4), and Malang-4 (V5)) were planted in two different locations which were Technical Implementation Unit Field Tenjo, Bogor (pH 4,8; 57 m asl) and Cikabayan Experimental field IPB, Dramaga, Bogor (pH 5,6; 200 m asl). Destructive observation was done at 4 months after planting to observe root length, tuber length, tuber diameter, and tuber mass. There was no significant difference between mutants and their parent lines on every observed variable. Number of leaves, stem diameter, tuber diameter, and tuber mass on every mutant and parent lines in acid upland were not significantly different with those planted in optimum upland. Plant height and tuber length in some mutants in acid upland were significantly higher than those planted in optimum upland. Disease incident of brown leaf spot (*Cercospora* sp.) was 90% and 47% and disease severity was 25-50% and 0-25% infected part of plant in acid upland and optimum upland respectively.

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Peer-review under responsibility of the organizing committee of LISAT-FSEM2015

Keywords: acid upland; morphology; tuber; yield characters

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1. Introduction

Cassava (*Manihot esculenta* Crantz) served as carbohydrate source for 800 million people in the world. In Indonesia, cassava was the third most important staple food after rice and maize. Productivity of cassava increased from 18.7 ton ha⁻¹ to 22.8 ton ha⁻¹ in 2009 and 2014 respectively. Even though there was an increase of cassava productivity and production, in 2014 Indonesia imported cassava of 24,558,778 ton in form of fresh and processed cassava [1]. It was evident that cassava production in Indonesia had not been able to fulfill the demand.

Cassava productivity in the last ten years was around 10 – 22 ton ha⁻¹ and its potential yield was around 25 - 40 ton ha⁻¹. Several cassava genotypes even had potential yield of 100 ton fresh tuber ha⁻¹ [2]. The gap between actual productivity and potential productivity was due to lack of superior seedling, under-optimum fertilization and pest and disease control, and cassava plantation on marginal land.

Marginal land in Indonesia was dominated by ultisol, which covers about 25% of total arable land in Indonesia. Ultisol was found mostly outside Java, making acid soil tolerant genotypes a crucial aspect in extensification effort.

Cassava breeding efforts was conducted to find cassava with high productivity, high starch content, and have tolerance for acid soil and high Al concentration in order to have cassava genotype that still have high yield under abiotic stress of marginal land. Preliminary effort of cassava breeding related to this research was done by Khumaida et al. [2] and Maharani [3] by irradiated five cassava genotypes using gamma rays to induce genetic variety to be selected. These cassava mutant lines were observed to find lines with desirable traits. This research was conducted to evaluate growth and performance of several potential M1V4 generation mutant in two different agro ecologies which were acid upland and optimum upland.

2. Methods

Stem cuttings from sixteen mutants and three parent lines (UJ-5 (V3), Adira-4 (V4), and Malang-4 (V5)) were planted in two different locations which were Technical Implementation Unit Field Tenjo, Bogor and Cikabayan Experimental field IPB, Dramaga, Bogor, West Java. Tenjo field had pH 4.8 soil and located at 57 m asl (above sea level), whereas Cikabayan field had pH 5.6 soil and located at 240 m asl. Fig. 1 showed maps of two locations. At each location, experimental design used was single factor completely randomized block design with five replications. Data from both locations was analyzed and if both data error was not significantly different, then data was analyzed as two factors, which were genotypes and locations.

Land preparation was done by making beds and application of manure of 5 ton ha⁻¹ two weeks before planting. Cassava cuttings with size of ± 20 cm (5 nodes) were planted with planting space of 1 m x 1 m. At two weeks after planting (WAP), fertilizer of 300 kg ha⁻¹ NPK (16-16-16) was applied. Second fertilizer application was given at 3 months after planting of 100 kg ha⁻¹ urea and 50 kg ha⁻¹ KCl.

Observation was conducted on growth parameter such as plant height, number of leaves, and stem diameter. Destructive observation was done at 4 months after planting to observe root length, tuber length, tuber diameter, and tuber mass.

3. Results

At the beginning of this experiment, Tenjo Field received moderate rain 85-115 mm per month, however Cikabayan received 116-150 mm per month. From 4 months after planting (MAP), both locations experienced moderate dry spell with 11 – 20 days without rain [4].

Genotypes planted at Cikabayan showed better growth than those planted at Tenjo in early growth (2 WAP). At 4 – 14 WAP, cassava planted at Tenjo had higher plant height than those planted at Cikabayan, as well as on number of leaves and stem diameter from 10-14 WAP (Table 1). Destructive observation results did not showed significant effect on root length between cassava planted at Tenjo and Cikabayan. On variable tuber length, diameter and mass, cassava planted at Tenjo had significantly higher value than those planted at Cikabayan (Table 2).



Fig. 1. Experiment locations map of Cikabayan experimental field and Tenjo experimental field.

Table 1. Location effect on plant height, number of leaves, and stem diameter.

Location	Variable	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP	12 WAP	14 WAP
Tenjo	Plant height	5.51	12.41	22.47	39.78	64.46	76.88	81.70
Cikabayan		6.22	10.22	18.07	33.27	38.82	47.83	53.00
t-value		*	**	**	**	**	**	**
Tenjo	Number of leaves	1.09	12.78	23.46	30.29	43.13	50.64	44.83
Cikabayan		3.87	15.09	22.32	31.79	36.38	37.78	37.70
t-value		**	**	ns	ns	**	**	**
Tenjo	Stem diameter	0.23	0.41	0.73	1.06	1.32	1.57	1.55
Cikabayan		0.30	0.40	0.67	1.04	1.04	1.17	1.18
t-value		*	Ns	*	ns	**	**	**

Note: t-value of *: significantly different based on DMRT with 5% alpha; **: significantly different based on DMRT with 1% alpha, ns: not significant based on DMRT; WAP: week after planting

Table 2. Location effect on root length, tuber length, diameter, and tuber mass of cassava at 4 MAP.

Location	Root length (cm)	Tuber length (cm)	Tuber diameter (cm)	Tuber mass (g)
Tenjo	20.34	18.33	2.69	56.03
Cikabayan	19.82	14.38	2.10	41.18
t-value	tn	**	**	**

Note: t-value of **: significantly different based on DMRT with 1% alpha, ns: not significant based on DMRT, MAP: month after planting

Cassava genotype gave significant effect on plant height, number of leaves, stem diameter, root length, and tuber length. Table 3 showed genotypes effect on plant height. Genotype respond observed from both location showed that there were genotypes with significantly better growth than others on almost every variable observed.

Observation on plant height showed that V5D2422 genotype had highest plant height at 8, 12, and 14 WAP and not significantly different with all genotypes of V5, V1D1523, and V1D212. On stem diameter variable, similar pattern was observed. Highest stem diameter was V5D2221 genotype and not significantly different with other genotypes of V5, V1D212, V4D0422, and V4D1133.

Table 3. Genotype effect on plant height of cassava at 8, 10, 12, and 14 WAP.

Genotype	Plant height (cm)			
	8 WAP	10 WAP	12 WAP	14 WAP
V3D0422	33.27 ghi	45.67bc	57.90 cdefg	57.75 de
V3D1222	34.47 efdh	48.45bc	63.07 abcdefg	68.20 abcd
V3D2413	37.32 cdefg	51.05bc	64.80 abcde	70.55 abc
V4D0433	34.92 defgh	48.82bc	61.60 bcdefg	67.40 abcd
V4D1133	33.42 fghi	46.45bc	58.17 cdefg	66.00 abcd
V4D1223	29.87 hi	46.45bc	55.25 defgh	58.85 cde
V4D1511	28.82 i	42.47bc	52.75 gh	58.36 cde
V4D2231	30.35 hi	44.15bc	53.50 fgh	58.50 cde
V4D2233	28.40 i	41.42bc	54.15 efgh	59.80 bcd
V5D0212	37.60 bcdefg	74.55a	64.20 abcdef	69.75 abcd
V5D1113	38.67 abcdefg	53.52bc	66.65 abc	71.65 ab
V5D1121	41.00 abc	57.22b	67.95 abc	69.10 abcd
V5D113	39.22 abcde	54.95bc	65.52 abcd	72.10 ab
V5D1223	40.05 abcd	54.2bc	64.60 abcde	69.80 abcd
V5D1333	43.4 a	56.97b	66.80abc	74.35 a
V5D2221	43.47 a	57.95b	72.40 ab	75.15 a
V5D2422	43.8 a	58.77b	72.85 a	76.55 a
V5D2423	39.22 abcde	53.42bc	62.70 abcdefg	68.82 abcd
V5D312	38.85 abcdef	55.63bc	66.50 abc	73.85 a

Note: Numbers followed by same letter were not significantly different based on DMRT with 5% alpha.

Table 4 showed that tuber diameter and tuber mass measured at first destructive observation (4 MAP) was not significantly different. Overall, genotypes of V5 and its mutants had higher root length, tuber length, tuber diameter, and tuber mass than other genotypes. Genotype V5D1121 had significantly higher tuber length than other genotypes.

Fig. 2 showed the quadrant matrix of number of tuber per plant and tuber mass per plant. In this figure, there were seven genotypes that have higher number of tubers and tuber mass per plant. There are significant interaction between location and genotype on plant height variable until 10 WAP. Interaction showed that there were genotypes that had different pattern between two locations. Input of LSMEANS was analyzed with DMRT to check the difference between treatment combinations.

Table 5 showed interaction between genotypes and locations analysis for plant height. Highlighted numbers were where the significant interaction found. Numbers without highlight meant that plant height on both locations was not significantly different. Observations at 12 – 14 WAP also did not showed significant difference on each genotype in both locations on plant height.

Table 4. Genotype effects on root length, tuber length, diameter, and tuber mass of cassava at 4 MAP

Genotype	Root length (cm)	Tuber length (cm)	Tuber diameter (cm)*	Tuber mass (g)*
V3D0422	16.25 cd	13.30 def	2.10	29.72
V3D1222	15.48 cd	16.58 bcd	2.25	42.30
V3D2413	13.66 d	14.08 bcd	1.83	26.70
V4D0433	17.36 bcd	16.25 bcd	2.31	42.57
V4D1133	16.83 bcd	17.16 bcd	2.33	40.52
V4D1223	20.47 abcd	19.53 b	1.88	34.92
V4D1511	20.86 abc	14.75 bcd	2.56	62.13
V4D2233	21.00 abc	12.02 f	2.66	28.64
V5D0212	25.58 a	13.41 cde	2.58	45.73
V5D1113	23.58 ab	18.70 bcde	2.38	38.88
V5D1121	23.88 ab	25.15 a	2.76	85.67
V5D113	20.51 abcd	16.81 bcd	2.61	47.32
V5D1223	21.7 abc	13.16 ef	2.74	62.56
V5D1333	22.41 abc	19.05 bcde	2.60	81.40
V5D2221	22.32 abc	19.37 bcd	2.61	47.81
V5D2422	22.41 abc	16.24 bcd	2.47	53.91
V5D2423	21.73 abc	16.66 bcd	2.85	48.45
V5D312	20.51 abcd	14.83 bcd	2.50	72.50

Note: Numbers followed by same letter were not significantly different based on DMRT with 5% alpha. *Not significantly different based on DMRT with 5% alpha.

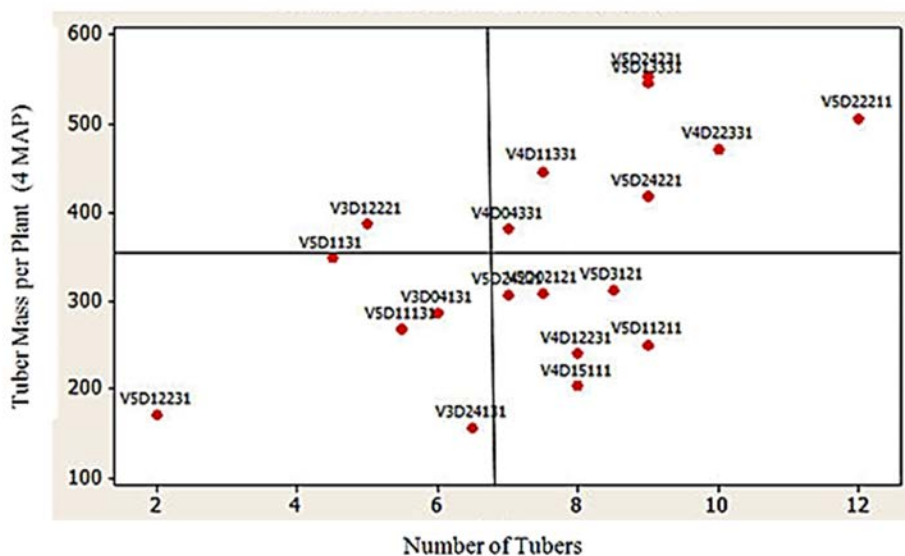


Fig. 2. The quadrant matrix of number of tuber per plant and tuber mass per plant.

Incident of brown leaf disease (*Cercospora* sp.) was counted from the number of infected plant divided by total number of plants. Disease incident observed at Tenjo was 90% and at Cikabayan was 47%. Disease severity was

counted by scoring which was 0 for 0%; 1 for 1-25%, 2 for 25-50%; 3 for 50-75%; and 4 for 75%-100% of infected leaf. Fig. 3 showed leaves infected with *Cercospora* sp. with score 1-4. Median value for disease severity at Tenjo was 2 and Cikabayan was 1. There were several genotypes that had lower disease severity (data not shown). These genotypes were V4D1133, V5D0, and V5D2422. Genotypes with high value of disease severity were V3D0 and V3D2413. Mutants that had higher disease severity in contrast with their background genotype were V5D1113, V5D1113, V5D1223, and V5D312.

Table 5. Interaction between genotype and location on plant height.

Genotype	Location	6 WAP		8 WAP		10 WAP	
V3D2413	Tenjo	24.95	abcde	40.25	abcdefg	61.70	bcdefghi
	Cikabayan	17.65	hijklmno	34.40	efgh	40.40	efghijkl
V4D1223	Tenjo	16.60	klmnopq	35.40	efgh	57.90	bcdefghij
	Cikabayan	13.55	mnopqr	24.35	jkl	27.30	l
V4D1511	Tenjo	19.44	efghijkl	33.55	fgh	57.00	bcdefghij
	Cikabayan	11.70	pqr	24.10	kl	27.95	kl
V4D2231	Tenjo	21.40	bcdefghijkl	35.70	efgh	57.40	bcdefghij
	Cikabayan	13.30	nopqr	25.00	ijkl	30.90	jkl
V4D2233	Tenjo	18.85	fghijklmn	35.55	efgh	55.60	bcdefghijk
	Cikabayan	10.80	R	21.25	l	27.25	l
V5D0212	Tenjo	25.95	abcd	44.45	abc	115.10	a
	Cikabayan	17.00	jklmnop	30.75	hijk	34.00	ijkl
V5D1113	Tenjo	26.30	abc	45.35	ab	69.45	bcd
	Cikabayan	18.45	fghijklmn	32.00	ghij	37.60	ghijkl
V5D1121	Tenjo	25.40	abcde	46.95	a	72.70	bc
	Cikabayan	21.50	bcdefghijkl	35.05	efgh	42.25	defghijkl
V5D2422	Tenjo	27.06	A	47.90	a	73.70	bc
	Cikabayan	21.85	bcdefghijkl	39.75	abcdefg	43.85	defghijkl
V5D2423	Tenjo	26.95	Ab	47.45	a	68.20	bcde
	Cikabayan	18.95	fghijklmn	31.00	hijk	38.65	fghijkl

Note: Numbers followed by same letter were not significantly different based on DMRT with 5% alpha. *Not significantly different based on DMRT with 5% alpha.



Fig. 3. Cassava leaves infected with *Cercospora* sp. with score 1-4 from left to right.

4. Discussion

Tenjo experimental field had low acidity soil (pH 4.8) and Cikabayan experimental field had more desirable acidity for crops (pH 5.6). When the soil pH drops below 5, Al^{3+} is solubilized into soil solution and this is considered to be

the most important limiting factor in acid soil. Acid soil usually also includes toxic level of manganese, and iron as well as deficiencies of several essential mineral elements.

However, the results from this experiment showed that the growth of cassava at acid upland was not significantly different with those planted in optimum upland. There were even several genotypes that had higher plant height on acid upland at the early growth. Aluminum toxicity primary respond was evident in roots, where roots become shorter a calloused [5]. This was not the case in this experiment. There was no significant difference in root length in both locations.

Plant in acid soils suffers from deficiencies in phosphorus, nitrogen, calcium, magnesium, and potassium. Severe phosphorus deficiency in cassava has thinner stems, shorter petioles, and narrower foliar lobes than do normal ones [6]. This was not observed in this experiment, since there was no significant difference on stem diameter in both locations. These could be a clear indication of these cassava genotypes tolerances on acid soil. Panda *et al.* [7] stated that there are two main classed of Al tolerance mechanism. Some plants operate to exclude Al from root apex and other are those that allow the plant to tolerate Al accumulation in the root and shoot symplasm. Further research need to be conducted to determined mechanism of Al tolerance in cassava.

Observation on growth parameters and destructive observations showed that there was no significant difference of cassava performance in acid upland and optimum upland. Cassava mutant lines and its background genotypes could be planted at acid upland without significant decrease on their growth. However, Susilawati *et al.* [8] analyzed chemical properties of Kasetsart variety cassava tubers from two locations, with soil pH 6 and pH 5. The results showed that difference in soil pH produced different tuber quality, where tuber from soil pH 6 had better starch content, water content, amylose content, and starch yield. Harvest from this experiment will be further examined to check chemical quality of tubers from both locations.

Observation on growth parameters showed that there were genotypes with better performance than others. Genotypes of V5 (Malang 4) and its mutant lines showed overall the best performance. Genotypes of V5 and its mutant lines, V4D0422, and V4D1133 had highest value of stem diameter. Khumaida *et al.* [2] and Maharani [3] showed that there were positive correlation between leaf colour and yield characters, and also stem diameter and tuber mass. It could be predicted that genotypes with bigger stem diameter would had higher tuber mass.

There was no significant difference on growth parameters between background genotypes with their mutant lines. This not necessarily meant that there were no genetics variety induced by gamma ray irradiation.

Disease incident and severity of brown leaf spot (*Cercospora* sp.) was higher on acid upland due to the difference in crops planted on both area. At the time experiment conducted, Tenjo field mostly was planted with farmer's cassava and papaya whereas Cikabayan field was planted with maize and fallow. The presence and severity of a plant disease is determined by the dynamic interaction of a susceptible crop (host), a causal agent (pathogen), and favorable environmental condition, or known as disease triangle [9]. Monoculture with low diversity of crops increasing the number of inoculums since the pathogen always have host to infect, thus more able to survive and spread. Cikabayan Field experiment was surrounded by fallow area and maize less likely to caused *Cercospora* sp. to spread because maize is not a host crop for *Cercospora* sp. resulting in lower disease incident and severity.

5. Conclusion

Cassava background lines (parent lines) and its mutant lines can grow well in acid upland and optimum upland. There were genotypes that showed better growth parameters which were genotypes of V5 (Malang 4) and its mutants. There was no significant difference between background lines and their mutant lines in all observed variables.

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